Phytochemical Analysis of Different Extracts of Nagaradi panchakaya

P. R. Waratenne1*, A. P. A. Jayasiri1, I. M. Manuha1 and P. M. H. K. Maduratharangi1

1Department of Ayurveda, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, 0800, Sri Lanka.

Authors’ contributions

This work was carried out in collaboration among all authors. Author PRW has designed the study, literature searches, wrote the first draft of the manuscript. Author APAJ has performed the chemical analysis and proof reading of the manuscript. Author IMM managed literature searches. Author PMHKM completed whole analysis part of the study. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Elmarie van der Watt, University of the Free State, South Africa.

Reviewers:
(1) Okoronkwo, Samuel Okafor, Ebonyi State University, Nigeria.
(2) D. Anusha, India.
(3) R. Badmanaban, Kerala University of Health Sciences, India.

Complete Peer review History: http://www.sdiarticle4.com/review-history/63181

Short Research Article

Received 18 September 2020
Accepted 23 November 2020
Published 05 December 2020

ABSTRACT

Nagaradipanchakaya is a very familiar decoction prescribed for respiratory tract diseases available in the Ayurveda Pharmacopoeia. It contains five herbal ingredients; i.e. Zingiber officinale, Cedrus deodara, Coriandrum sativum, Solanum indica and Solanum xanthocarpum.

Aims: The study was conducted to analyze the phytochemicals present in sequential extracts of Nagaradipanchakaya using hexane, ethyl acetate and ethanol solvents in succession.

Place and Duration of Study: The study was conducted in the unit of Dravyaguna Vignana, Department of Ayurveda, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka, between October to December 2019.

Methodology: Authentication of the ingredients of Nagaradipanchakaya was done by the experts in the Unit of Ayurveda Pharmacology in the Institute of Indigenous Medicine, University of Colombo. The extracts of dried powder of the ingredients obtained using Hexane, Ethyl acetate and Ethanol in succession were tested along with blank (water) for the presence of phytochemicals. Chemical analysis carried out for (a) qualitative analysis of phytochemical constituents (b) quantitative analysis of alkaloids, total phenol, flavonoid and Saponin contents.

*Corresponding author: Email: drwaratenne@gmail.com;
### Results:
The results suggested that the presence of Alkaloids, Tannin and Phenolic compounds, Terpenoids & steroids, Flavonoids, Cardiac Glycosides, Saponins, Carbohydrates, Amino acids, Anthraquinone glycosides, Resins having selective solubility in selected solvents of varying polarities used in succession. The selective solubility of the phytochemicals is probably responsible in conferring a wide spectrum of biological activities attributed to the decoction of Nagaradi Panchakaya. In addition, the present study suggests that the sequential extractions using solvents of varying polarities would maximize the exploitation of the diverse bioactive compounds. Further considerable amount of Alkaloids (3.52 ± 0.15%), phenolic compounds (47.03±0.56 mg gallic acid equivalents), flavonoids (15.83±0.16 mg quercetin equivalents/g and Saponin (4.1±0.4%) were present in this poly-herbal formula.

**Conclusion:** The present study revealed the presence of phytochemicals such as alkaloids, tannin, phenolic compounds, terpenoids & steroids flavonoids, tannins and saponins in this Nagaradipanchakaya.

**Keywords:** Nagaradipanchakaya; phytochemicals; alkaloids; phenolic compounds; flavonoids.

### 1. INTRODUCTION

*Nagaradipanchakaya* is a well-known and frequently prescribed decoction for respiratory tract diseases by Sri Lankan Ayurveda medical practitioners available in the Ayurveda Pharmacopoeia [1]. It contains five ingredients; so it is named *Paspanguwa* as well. The ingredients are *Zingiber officinale*, *Cedrus deodara*, *Coriandrum sativum*, *Solanum indicus* and *Solanum xanthocarpum*. Tremendous research works confirmed the presence of phytochemicals of these ingredients separately; but there is a big gap on phytochemical analysis of this polyherbal formula/mixture of herbs.

In this study three solvents were selected according to increasing the polarity because different chemical constituents can be extracted to the media based on the polarity. Therefore in order to extract varying range of phytochemicals we used different solvents of non-polar, mid-polar and polar category of solvents in successive /sequential manner to investigate which types of the solvent extract get more phytochemicals.

The aim of the present study was to reveal the qualitative analysis of phytochemicals present in different solvents extracts of the *Nagaradipanchakaya* and quantitative analysis of selected phytochemicals present in this mixture of herbs.

### 2. MATERIAL AND METHODS

#### 2.1 Collection and Identification of the Plant Materials

The plant materials such as the rhizomes of *Inguru* (*Zingiber officinale*), stem pieces of *Devadara* (*Cedrus deodara*), seeds of *Kottamalli* (*Coriandrum sativum*), roots of *Batu* (*Solanum indicus*) and total plant of *Katuwellabatu* (*Solanum xanthocarpum*) were purchased from local Ayurveda medical shops in Colombo city (6°55′54.98″ N x 79°50′52.01″E), Western province, Sri Lanka and were identified by the senior lecturer in the unit of Ayurveda Pharmacology of the Institute of Indigenous Medicine, University of Colombo.

#### 2.2 Preparation of Sample Extracts (Solvent Extraction)

To prepare the hexane, ethyl acetate and ethanol extracts, first the materials were sorted out by removing diseased parts, the unnecessary parts and were washed under the running tap water to remove soil and other dust particles. Then the samples were air dried separately under the laboratory condition for 2-3 weeks.100 g of each of the five ingredients of *Nagaradipanchakaya* were weighted and ground to a fine powder using grinder(Disk Mill Model FFC-234, China), separately. 100g of each powder of the above ingredients were mixed and put into a conical flask and solvent was added and kept for 24 hours and a continuous extraction was done with hexane, ethyl acetate and ethanol (95%) respectively. Each extract obtained following successive extraction was filtered using Whatman No. 1 filter papers. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 40°C. Finally the crude was taken into vials, labeled and was stored in a freezer at -4°C in the refrigerator, till further use.

#### 2.3 Preparation of Reagents [2]

##### 2.3.1 Mayer’s reagent

Amount of 1.356 g of HgCl$_2$ was dissolved in 60 ml of distilled water and the second solution was...
prepared by dissolving 5 g of KI in 10 ml of distilled water. Then the two solutions were mixed together and final solution was diluted to 100 ml.

2.3.2 Hager’s test reagent
This was prepared by dissolving 1 g of Picric acid in 100 ml of distilled water.

2.3.3 Wagner’s test reagent
Amount of 12.5 g of KI and 2.5 g of iodine were dissolved in 100 ml distilled water and it was diluted to 250 ml.

2.3.4 Fehling’s a solution
Fehling’s A solution was prepared by dissolving 6.8 g of CuSO4 in 100 ml distilled water and 2-3 drops of H2SO4 was added and mixed well.

2.3.5 Fehling’s b solution
Fehling’s B solution was prepared by dissolving 34.8 g of Sodium potassium tartrate and 5 g of NaOH in 100 ml distilled water.

2.3.6 Benedict’s reagent
One solution was prepared by dissolving 7.3 g of Sodium citrate and 10 g of anhydrous sodium carbonate in 80 ml of distilled water. Second solution was prepared by dissolving 1.75 g of CuSO4 in 10 ml of distilled water. Then the two solutions were mixed with rapid stirring and it was diluted to 100 ml.

2.3.7 Seliwanoff’s reagent
This was prepared by dissolving 0.1 g of Resorcinol in 100 ml of concentrated HCl and then it was diluted up to 250 ml.

2.3.8 Ninhydrine solution
Amount of 2 g of Ninhydrin was dissolved in 100 ml of acetone.

2.3.9 Millon’s reagent
Amount of 10g of Hg was dissolved in 10 ml of fuming nitric acid and 40 ml of distilled water was added.

2.4 Qualitative Analysis of Phytochemicals
A stock concentration of 1% (W/ V) of each successive extract obtained using hexane, ethyl acetate and ethanol was prepared using the respective solvent. These extracts along with negative controls were tested for the presence of active phytochemicals. All the tests were conducted using standard methods described in the journal articles mentioned below. All the tests were triplicated and necessary minor modifications in the tests were done accordingly.

2.4.1 Tests for alkaloids
In this study, three (3) different tests such as Mayer’s test, Hager’s test and Wagner’s test were performed to be identified the alkaloids presence in the three different solvent extracts.

2.4.1.1 Mayer’s test
To 2 ml of each extract solutions (test solutions), were treated with a few drops of the Mayer’s reagent. Formation of pale yellow / white colour precipitate confirm the existence of alkaloids [3,4].

2.4.1.2 Hager’s test
To 2 ml of each extract solutions (test solutions), a few drops of the Hager’s test reagent was added. The presence of pale yellow precipitate confirms the test as positive [3,4].

2.4.1.3 Wagner’s test
To 2 ml of each extract solutions (test solutions), a few drops of the Wagner’s test reagent with 2-3 drops of concentrated HCl were added. The formation of brown precipitate indicates the presence of alkaloids [3,4].

2.4.2 Tests for tannins and phenolic compounds
Three (3) different tests such as Ferric chloride test, Gelatine test and Lead acetate test were performed to identify tannins and phenolic compounds presence in the three different solvent extracts.

2.4.2.1 Ferric chloride test
To 2 ml of each extract solutions (test solutions), were treated with a few drops of 5% ferric chloride. The formation of deep blue / dark green precipitate indicates the presence of tannins and phenolic compounds [5].

2.4.2.2 Gelatine test
Two (2) ml of each extract solutions (test solutions), were treated with a few drops of 10%
sodium chloride solution and a few drops of 1% w/v gelatine solution. The formation of white precipitate confirms the presence of tannins and phenolic compounds [5].

2.4.2.3 Lead acetate test

To 02 ml of each extract solutions (test solutions), a few drops of 10% w/v lead acetate solution was added. The formation of bulky white precipitate indicates the presence of tannins and phenolic compounds [6].

2.4.3 Tests for terpenoids and steroids

In this study Salkowski and Libermann Burchard’s tests were performed to identify the terpenoids & steroids, presence in the three different solvent extracts.

2.4.3.1 Salkowski test

To 2 ml of each extract solutions (test solutions), 1 ml of concentrated sulphuric acid was added. The appearance of red colour in lower layer indicates the presence of steroids and yellow colour in lower layer indicates the triterpenoids [4].

2.4.3.2 Libermann burchard’s test

A portion of 10 mg of dried extraction was dissolved in 8 ml acetic anhydride and prepared test solution. 2 ml of each test solutions were boiled separately and allowed to cool and then 1 ml of concentrated H$_2$SO$_4$ was added along the wall of the test tubes. The appearance of brown colour ring at the interface and upper layer turns to green colour indicates the presence of steroids. If upper layer turns to deep red colour indicates the presence of triterpenoids [4].

2.4.4 Tests for flavonoids

Four (4) different tests such as Amonia test, Alkaline test, Lead acetate test and Shinoda test were performed to identify flavonoids in three different solvent extracts.

2.4.4.1 Ammonia test

2 ml of each extract solutions (test solutions), were treated with 2 ml of dilute ammonia (10%) and a few drops of concentrated H$_2$SO$_4$. The appearance of yellow orange colour with the addition of dilute ammonia and the disappearance of that colour after some time indicates the presence of flavonoids [6].

2.4.4.2 Alkaline test

2 ml of each extract solutions (test solutions), were treated with a few drops of 5% NaOH solution and a few drops of dilute HCl were added. The appearance of intense yellow colour with the addition of a few drops of 5% NaOH solution and this yellow colour becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids [3,6,7].

2.4.4.3 Lead acetate test

2 ml of each extract solutions (test solutions), were treated with a few drops of 10% Lead acetate solution. The presence of bulky white precipitate confirmed the positive results of glycosides [3,6].

2.4.4.4 Shinoda test

1 ml of concentrated HCl and a few pieces of magnesium was added into 2 ml of each extract solutions (test solutions). Disappearance of the colour of the solution with the addition of 1 ml of HCl and the appearance of pink or red colour with the addition of a few pieces of magnesium indicates the presence of flavonoids [3].

2.4.5 Tests for cardiac glycosides

Two (2) different tests such as Legal test and Keller Killiani test were performed to identify Cardiac glycosides in three different solvent extracts.

2.4.5.1 Legal test

2 ml of pyridine and 1 ml of alkaline sodium nitroprusside solution were added to 2 ml of each solvent extract solutions (test solutions). The appearance of pink to red colour in the solution confirms the presence of glycosides [4].

2.4.5.2 Keller killiani test

To 2 ml of each aqueous solution (test solution), a few drops of glacial acetic acid and a few drops of concentrated sulphuric acid and a trace amount (01 drop) of Ferric chloride solution were added. The appearance of reddish brown colour ring at the interface and upper layer turns to greenish colour indicates the presence of glycosides [4,8].

2.4.6 Test for saponins

Two (02) different tests such as Foam Test and Olive oil Test were performed to identify Saponins.
2.4.6.1 Foam test

05 ml of each extract solutions (test solutions), shaken vigorously until form a stable persistent foam [9].

2.4.6.2 Olive oil test

The above froths were mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion should be observed [9].

2.4.7 Tests for carbohydrates

Three (3) different tests, such as Fehling's test, Benedict's test and Seliwanoff's test were performed to identify Carbohydrate.

2.4.7.1 Fehling’s test

1 ml of each Fehling’s A and Fehling’s B were heated for 1 minute. Heated Fehling’s solution was added to 2 ml of each extract solutions (test solutions). Then, this was heated for 2 minutes in a water bath. The appearance of brick red precipitate indicates the presence of carbohydrate [4,5].

2.4.7.2 Benedict’s test

To 2 ml of each extract solutions (test solutions), 1 ml of Benedict’s reagent was added and heated for 2 minutes in a water bath. The appearance of red colour precipitate indicates the presence of carbohydrate [4,5].

2.4.7.3 Seliwanoff’s test

To 2 ml of each extract solutions (test solutions); 1 ml of Seliwanoff’s reagent was added. Then it was heated for 2 minutes in a water bath. The appearance of rose colour precipitate indicates the presence of carbohydrate [5].

2.4.8 Tests for amino acids

Two (2) different tests such as Ninhydrine test and Millon’s test were performed to identify Amino Acids.

2.4.8.1 Ninhydrine test

To 2 ml of each extract solutions (test solutions), Ninhydrine solution was added and then heated for 2 minutes in a water bath. The appearance of blue or violet in the solution indicates the presence of amino acid [10].

2.4.8.2 Millon’s test

To 2 ml of each extract solutions (test solution), 1 ml Millon’s reagent was added and then the final setup was heated for 2 minutes. The appearance of white colour precipitate with the addition of 1 ml Millon’s reagent and the colour turn to red with heating indicates the presence of amino acid [10].

2.4.9 Test for anthraquinone glycosides

Two (2) different tests such as Hydroxyanthraquinone test and Borntrager’s test were performed to identify Anthraquinone glycosides.

2.4.9.1 Hydroxyanthraquinone test

To 2 ml of each extract solutions (test solutions); 1 ml 10% potassium hydroxide was added. The appearance of red colour in the solutions indicates the presence of hydroxyanthraquinone [3].

2.4.9.2 Borntrager’s test

To 2 ml of each aqueous solution (test solution), 2 ml dilute sulphuric acid was added. Then it was boiled and filtered and allowed to cool. To the filtrate equal volume of chloroform was added and shakes well. The organic solvent was separated and ammonia solution was added. The appearance of pink or red colour in the ammonia layer indicates the presence of hydroxyanthraquinone glycosides [4,8].

2.5 Quantitative Analysis of Phytochemicals

Five ingredients of Nagaradipanchakaya were washed under the running tap water to remove soil and other dust particles and air dried separately under the laboratory condition for 2-3 weeks. Then 20 g of each ingredient were weighted and separately ground to a fine powder using grinder (Disk Mill Model FFC-234, China) and mixed together. Quantitative analysis of each phytochemical was done in triplicate and average value was calculated as mentioned below.

2.5.1 Quantitative determination of alkaloids

Quantitative determination of alkaloid was done according to the methodology described by Harborne [11]. Two hundred milliliters of 10% acetic acid in ethanol was added to each sample
(approx. 2.50 g) in a 250 ml beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 30 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation was complete immediately after filtration. After 6 hours of the mixture sedimentation, the supernatant was discarded and the precipitates were washed with 50 ml of 0.1M of ammonium hydroxide and then filtered using filter paper dried in a oven for 4 hours at 105°C. Using electronic weighing balance, the residue was dried in oven and the percentage of alkaloid is expressed mathematically as:

\[
\% \text{ Alkaloid} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample}}
\]

### 2.5.2 Quantitative determination of total polyphenolic content

The total polyphenolic content was estimated according to the Folin – Ciocalteu method [12].

Known concentrations (0 – 100 mg/ml; n = 3) of hot water extract of the sample (0.1 ml) or gallic acid (0.1 ml) were diluted with distilled water (0.9 ml). To each tube 5 ml of a 10 fold diluted solution of Folin – Ciocalteu reagent was added and mixed. Four milliliters of saturated sodium carbonate solution was added to each of the above tubes and the mixture shaken. After 2 h, the absorbance of each reaction mixture was measured at \(\lambda\) 765 nm. Gallic acid was used as the standard for the calibration curve. Total phenolic content was calculated on the basis of the calibration curve of gallic acid and the results were expressed as mg of gallic acid equivalents per g of sample (mg gallic acid/g sample).

### 2.5.3 Quantitative determination of total flavonoid content

The total flavonoid content was determined using the Dowd method [13]. In this experiment, 5 ml of 2% AlCl3 in methanol was mixed with the same volume of hot water extract of the sample or quercetin in known concentrations (0 – 100 mg/ml; n = 3). After 10 min. the absorbance of the reaction mixture was measured at \(\lambda\) 415 nm. Quercetin acid was used as the standard for the calibration curve. Total flavonoid content was calculated on the basis of the calibration curve of quercetin and the results were expressed as mg of Quercetin equivalents per g of sample (mg quercetin /g sample).

### 2.5.4 Quantitative determination of saponin content [14,15]

Exactly 100 ml of 20% aqueous ethanol was added to 5 g of sample in a 250 ml conical flask. The mixture was heated over a hot bath for 4 hours with continues stirring at a temperature of 55°C. The residue of the mixture was re-extracted with another 100 ml of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55 °C with constant stirring. The combined extract was evaporated to 40 ml over water bath at 900°C. Diethyl ether (20 ml) was added to the separator funnel and vigorously agitated from which the aqueous layer was recovered while the ethyl layer discarded. This purification process was repeated twice. 60 ml of n-butanol was added and extracted twice with 10 ml of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to constant weight. The saponin content was calculated as percentage:

\[
\% \text{ Saponin} = \frac{\text{Weight of saponin} \times 100}{\text{Weight of sample}}
\]

### 3. RESULTS AND DISCUSSION

#### 3.1 Qualitative Analysis of Phytochemicals

The results obtained were graded as very high (+++); high (++); moderate (+); low (+) and nil (-) based on the intensity of the colored reaction product of the test compared to each other in each test are shown in the Table 1. The detailed investigations of phytochemicals in various solvents are shown in the Figs. 1-9.

The results of the phytochemical analysis of the successive extracts of *Nagaradipanchkaya* in various solvents along with blank have shown a remarkable variation. The results revealed that presence of alkaloids was confirmed by three tests in all three extracts, but maximum presence in ethanol extract. Presence of Tannins & Phenolic compounds was confirmed by three tests in ethyl acetate extract and ethanol extract but only one test confirmed in Hexane extract. Terpenoids and steroids were confirmed by three tests in all extracts but maximum in ethanol extract. Flavonoids were confirmed in ethyl acetate extract and ethanol extract but only one test confirmed in Hexane extract. Cardiac
Table 1. Levels of phytochemicals in the successive extracts of *Nagaradi panchakaya* along with blank (water)

<table>
<thead>
<tr>
<th>Phytochemical Tests</th>
<th>Blank</th>
<th>Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayer’s Test</td>
<td></td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Hager’s Test</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Wagner’s Test</td>
<td></td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td><strong>Tannins &amp; Phenolic compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeCl₃ Test</td>
<td></td>
<td></td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Gelatin Test</td>
<td></td>
<td></td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Lead acetate Test</td>
<td></td>
<td>+</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td><strong>Terpenoids and steroids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Salkowski Test (Steroids) | | | | ++++
| Libermann Burchard’s Test | | | | +++
| **Flavonoids**      |       |                |                       |                 |
| Ammonia Test        |       |                | ++                    | +++             |
| Alkaline Test       |       |                | ++                    | +++             |
| Lead acetate Test   |       | +              | +++                   | ++++            |
| Shinoda Test        |       |                | +                     | +++             |
| **Cardiac Glycosides** | | | | |
| Legal Test          |       | +              | ++                    | ++++            |
| Killer Killiani Test|       |                | +                     | ++++            |
| **Saponins**        |       |                |                       |                 |
| Foam Test           |       |                |                       | +++             |
| Olive oil Test      |       |                |                       | +++             |
| **Carbohydrates**   |       |                |                       |                 |
| Fehling’s Test      |       |                |                       | +++             |
| Benedict’s Test     |       |                |                       | ++             |
| Seliwanoff’s Test   |       |                |                       | +              |
| **Amino Acids**     |       |                |                       |                 |
| Ninhydrine Test     |       |                |                       | ++++            |
| Milk’s Test         |       |                |                       | ++             |
| **Anthraquinone glycosides** | | | | +++
| Hydroxy anthraquinone Test | | | | +
| Borntrager’s Test   |       |                |                       | +              |
| **Resins**          |       |                |                       |                 |
| Test for resins     |       |                | +++                   | ++++            |

The values are means of three replicates with standard deviations (mean ± S.D)

Glycosides confirmed by three tests but maximum in ethanol extract. Saponins was not confirmed in Hexane and ethyl acetate extracts but confirmed in ethanol extract by two tests. Carbohydrates was not confirmed by three tests for Hexane extract but was confirmed by two tests of ethyl acetate extract and three tests of ethanol extract and they were maximum. Amino Acids was not confirmed by two tests of hexane extract but confirmed by one test of ethyl acetate extract and two tests of ethanol extract. Anthraquinone glycosides were confirmed only in ethyl acetate extract and ethanol extract but maximum in ethanol extract. Resins were confirmed in all extracts.

Table 2. Total alkaloid, phenolic, flavonoid and saponin content of *Nagaradipanchakaya*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemicals</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total alkaloid percentage</td>
<td>3.52 ±0.15</td>
</tr>
<tr>
<td>2.</td>
<td>Total phenolic content in mg of gallic acid equivalents/g</td>
<td>47.03±0.56</td>
</tr>
<tr>
<td>3.</td>
<td>Total flavonoid content in mg of quercetin equivalents/g</td>
<td>15.83±0.16</td>
</tr>
<tr>
<td>4.</td>
<td>Total saponin percentage</td>
<td>4.1 ± 0.4</td>
</tr>
</tbody>
</table>

The values are means of three replicates with standard deviations (mean ± S.D)

3.2 Quantitative Determination of Total Alkaloid, Phenolic, Flavonoid and Saponin Content

The results revealed that considerable amount of Alkaloid, Phenols, Flavonoid and Saponin were present in the herbal preparation as shown in the Table 2.

R² for total phenols and flavonoids were 0.9990 and 0.9890 respectively.

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to
Tannins are also involved in treatment of carcinogenic and anti-inflammatory effects exhibit anti-hypoglycaemic, anti-allergic, anti-inflammatory, anti-cancer and anti-carcinogenic properties. Tannins are also involved in treatment of non-insulin dependent diabetes mellitus by enhancing the glucose uptake and inhibiting adipogenesis [21]. In this study revealed the presence of Alkaloids, Tannin and Phenolic compounds, terpenoids & steroids, Flavonoids, Cardiac Glycosides, Saponins, Carbohydrates, Amino acids, Anthraquinone glycosides and Resins which is having such pharmacological actions specially in the ethanol extract of Nagaradi panchakaya. Presence of considerable amount of alkaloids, phenolic compounds, flavonoids and saponins further confirm the medicinal values of this poly-herbal formula.

4. CONCLUSION

Study revealed the presence of phytochemicals such as Alkaloids, Tannin and Phenolic compounds, terpenoids & steroids, Flavonoids, Cardiac Glycosides, Saponins, Carbohydrates, Amino acids, Anthraquinone glycosides and Resins in all the extracts of the Nagaradi panchakaya, and they were more dominant in ethanol extract.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

Authors would like to acknowledge financial support from the Institute of Indigenous medicine, University of Colombo, Sri Lanka and Dr. L.D.A.M. Arawwawala, Industrial Technology Institute, Sri Lanka for the assistance in chemical analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

3. Roopalatha UC, Mala V, Nair G. Phytochemical analysis of successive re-
4. Joshi A, Bhobe M, Sattarkar A. Phytochemical investigation of the roots of
5. Saklani S, Mishra AP, Sati B, Sati H. Pharmacognostic, phytochemical
and antimicrobial screening of aphanamixispolyystachya, an endangered
phytochemical analysis of ethanolic extracts of leaves of olea dioica roxb.,
infected with the rust fungus zaghouania oleae (E. J. Butler) cummins and
7. Shah P, Modi HA, Shukla MD, Lahiri SK. Preliminary phytochemical analysis and
antibacterial activity of ganodermafulvicum collected from dang
8. Iqbal E, Abu K, Lim LBL. Phytochemical screening, total phenolics
and antioxidant activities of bark and leaf extracts of goniobotryum simplex
9. Edeoga HO, Okwu DE, Mbaebe BO. Phytochemical constituents of some
10. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal
11. B Harborne, Phytochemical methods: A
guide to modern techniques of plant
12. Singleton VL, Orthofer R, Lamuela -Raventos RM. Analysis of total phenols
and other oxidation substrates and antioxidants by means of Folin-Ciocalteu
phenolic, flavonoid and proline contents in burkina fasan honey, as well as their
14. Ejikeme CM, Ezeonu CS, Eboatu AN. Determination of physical and
phytochemical constituents of some tropical timbers indigenous to Niger Delta
15. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the
crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global
16. Holst B, Williamson G. Nutrients and phytochemicals: From bioavailability to bio
17. Ayurveda Pharmacopoeia Committee -
Department of Ayurveda. Ayurveda
Pharmacopoeia. 1st ed. Maharagama – Sri
18. Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G. Phytochemistry of
Medicinal Plants. Journal of Pharmacogn
82.
19. Sandhar HK, Kumar B, Prasher S et al. A
Review of phytochemistry and pharmacology of flavonoids. Int Pharma
20. Al-Shahwany AW. Alkaloids and phenolic
compound activity of piper nigrum against
some human pathogenic bacteria. Biomed
with positive effect to manage diabetes.